

SEASONAL VARIATION OF SURVIVAL TIME IN ANOXIC WATER AND THE GLYCOGEN CONTENT OF *SPHAERIUM CORNEUM* AND *PISIDIUM AMNICUM* (BIVALVIA, PISIDIIDAE)

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ABSTRACT

I surveyed the ability of two freshwater bivalves, *Sphaerium corneum* (L.) and *Pisidium amnicum* (O. F. Müller), to survive anoxic water of different temperatures. Experiments using a small volume (2 to 5 ml water per 5-8 mm clam), closed-bottle method were run for over one year on individuals from small water bodies in eastern Finland. Total amount and location of glycogen in these bivalves was also examined by chemical analyses and histochemical methods.

Both species showed good ability for anaerobiosis. The 50% survival time for *Pisidium amnicum* was ca. 4.5 days at 20°C, but increased with decreasing temperatures, being almost 200 days at 0°C. *Sphaerium corneum* survived even better, with the corresponding survival times roughly twice as long at all temperatures. The results suggested that seasonal variation is not deducible from temperature alone.

Glycogen content of both species in nature varied seasonally between 0.5 and 3% glycogen per wet weight of tissue, with peak values attained in late autumn and early summer. The habitat of *Pisidium amnicum* was well oxygenated during winter, although part of the population overwintered in anoxia in ice or frozen sediment. The decrease in glycogen content during winter, and the seasonal variation in general, were more pronounced in clams experiencing anoxia. Ecological consequences of this anaerobic capacity and potential effects on the results of energetic studies are suggested to be important.

Small lakes and ponds in cold temperate areas commonly experience winter anoxia (e.g. Nagell and Brittain, 1977; Salonen *et al.*, 1984). This anoxia starts from the bottom sediments and a steep microstratification may often be found in the overlying water.

Consequently euryoxy, or capacity for facultative anaerobiosis (or even obligate, as in *Tubifex*, Famme and Knudsen, 1985), is common in benthic animals (Eggleton, 1931; Lindeman, 1942; Beadle, 1961; Seuss *et al.*, 1983). Examples include both marine and freshwater molluscs (De Zwaan, 1977, 1983; Gäde, 1983; Kluytmans and Zandee, 1983).

The tiny freshwater bivalves of the family Pisidiidae are relatively inactive, slow-moving infaunal animals. Since they are capable of neither "burst"-activity nor long migrations they are confined to certain areas of bottom sediment throughout their lives. As a result they must be able to tolerate all of the environmental variation present in these habitats, including anoxia. In addition to survival in anoxic water, a good capacity for anaerobiosis is of obvious value for

Pisidiidae in aerial exposure, e.g. during passive dispersal (Mackie, 1979), aestivation in drying mud (Bleck and Heitkamp, 1980; McKee and Mackie, 1980) or wintering in ice (Olsson, 1981). Earlier reports of anoxia tolerance in Pisidiidae were given by Juday (1908), Jatzenko (1928), Eggleton (1931), Thomas (1963, 1965), Gale (1976), Way *et al.* (1980), Burky (1983) as well as Holopainen and Jónasson (1983). In addition, Dietz and Stern (1977) demonstrated a seasonal variation in carbohydrate content of *Sphaerium transversum* (Say). The aims of this study were to experimentally survey the ability of two common pisidiid species, *S. corneum* and *Pisidium amnicum*, to survive in anoxic water at different temperatures and in different seasons, and to examine the possible role of glycogen in this ability.

MATERIALS AND METHODS

Material was collected between August 1984 and August 1985 from the Siilaispuro River (*Pisidium amnicum*), and Lake Varaslampi (*Sphaerium corneum*), both situated in

the town of Joensuu, in eastern Finland (62°37' N, 29°45' E). The river is about 3-4 m wide and 1.5-2.0 m deep at the sampling site. *P. amnicum* lives on the slopes in the soft bottom of mud and plant litter. Lake Varaslampi is oval with an area of about 3 ha and a maximum depth of about 4 cm. It is fringed by lush vegetation and has a thick mud deposit (4 m) in the deepest part. *S. corneum* was collected from the vegetation belt around the lake (open water period) and from the outlet ditch (during ice cover), where the population density was much higher than in the lake.

Both the river and lake are ice covered approximately 5 months (November - May) annually and have an annual temperature variation from 0.5 to 22°C (Fig. 1). Physical and chemical properties of the water are listed in Table 1. The oxygen content of the river water was high year round (min. 74% of saturation in March), whereas a severe depletion of oxygen in the lake caused a minor fish kill in April 1985. Total anoxia developed at a depth of 4 m in early December and reached the surface water in March. The sediment in the lake littoral probably experienced anoxia for 1-2 months during 1984-1985 winter. The outlet ditch stayed unfrozen for about 2 weeks in autumn and thawed again about 3 weeks earlier in spring, which may have considerably improved the oxygen availability in this site.

Both species were sampled by hand net at about 1 month intervals (with a 3 month pause in mid-winter). Samples were sorted and experiments were started on the same or following day. Adult clams of various sizes (5-8 mm) were placed in glass jars. River or lake water with very low oxygen content (< 0.5 mg/l) was then added. Bubbling with pure nitrogen for 1 hour was used to lower the oxygen content down to 0.5 mg/l or less. The volume of water per clam varied from 2 to 5 ml (5 to 10 clams in each jar with volume of 10-50 ml). The jars were then sealed with tight rubber or ground glass stoppers, covered with aluminium foil and immersed into constant-

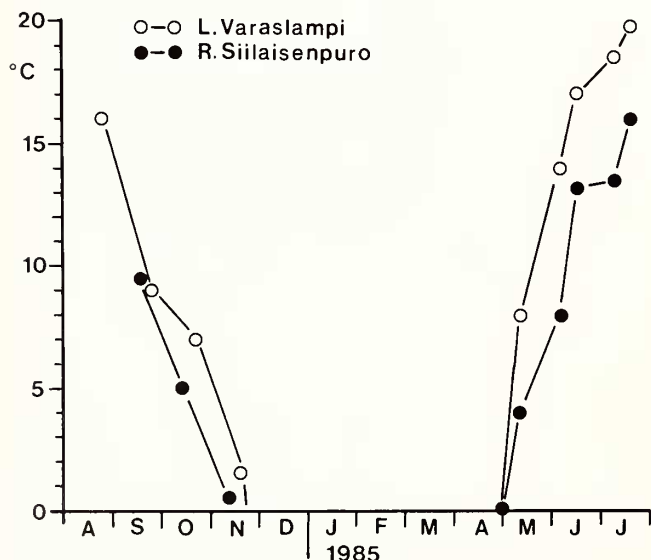


Fig. 1. The temperature of surface water in Lake Varaslampi and Siilaisempuro River in the sampling dates.

Table 1. Mean values of some physical and chemical properties of water in the study sites.

		Siilaisempuro River	Lake Varaslampi
Conductivity	μmho/cm	110	220
pH		6.5	6.8
Color	Pt mg/l	110	70
COD _{Mn}	mg/l O ₂	9.5	9.5
Tot. P	μg/l	106	75
Tot. N	μg/l	850	900
Ca	mg/l	12	25

temperature baths. Every 1 to 4 days the jars were examined for dead animals. A clam was considered dead when its shell valve was open and the animal did not react to a shaking of the jar by closing its shell or withdrawing its foot. In addition, the heart beat of younger clams could be seen (and heart rate measured) through the glass by using a stereomicroscope and transmitted light. Initially, open vials were used as controls but because of almost no mortality in them, use of controls was later discontinued.

Five to 15 clams were damp-dried on tissue paper, put into glass jars and stored deep-frozen at -40°C. The glycogen content of each individual was determined later by the method of Siu *et al.* (1970) and expressed as per cent of tissue wet weight (WW) (shell excluded). These can be converted to approximate values per tissue dry weight (DW) by multiplying by a factor of 8 for *Pisidium amnicum* (water 55% of WW and ash 85% of DW in intact clam) and 13 (water 72%, ash 80%) for *S. corneum*.

Additional clams were fixed in alcoholic Bouin, treated by the customary wax-embedding method, sliced and stained by Lillie's (1951) allochrome procedure to reveal glycogen concentrations.

For comparison, identical experiments were performed on samples from three additional populations of *Pisidium*. *Pisidium casertanum* and *P. subtruncatum* Malm were collected in September 1984 from 20 m in eutrophic Lake Esrom, Denmark, and *P. amnicum* from 0.5 m in oligotrophic Lake Pääjärvi were collected in March, May and June 1985.

In the Siilaisempuro River, seasonal water level fluctuation caused the ice to contact the sediment during winter. In very shallow areas the sediment surface froze tightly to the ice. From this area (20-30 cm of water at the time of freezing) two ice clumps (680 and 1600 cm²) were removed in March by chainsaw, and the frozen loose sediment on the bottom of the ice was rinsed and scraped away. The 30 cm thick pieces of solid ice with about 5 cm of frozen sediment enclosed were taken to the laboratory and thawed at 5°C. The material was then sieved and examined for living *Pisidium amnicum*.

RESULTS

BEHAVIOUR

The first reactions to sudden immersion in anoxic water

were foot extension and increased locomotory activity. Within a few hours this changed to total inactivity and a tightly closed shell. *Sphaerium corneum* had longer periods of activity and, during the first few days, was often observed to crawl up to the roof of the vial. The clam then attached itself to the roof by a slime thread protruding from the middle of the foot slit and stayed hanging up. On several occasions this species also floated up in the vial, probably by a bubble (of unknown gas and origin) inside the shell. During the main period of anoxia pedal activity was rare and never the result of the shaking of the vial when regularly checked. On the other hand, an increase in temperature of several degrees (due to temporary power failures that occurred twice during winter) caused extension of the foot for some time in both species.

Even when heart rate was variable and sensitive to disturbance, the results suggested clear bradycardia during anoxia. At 9° to 16°C the usual aerobic rate was 20-30 beats per minute in small *Sphaerium corneum*, *Pisidium casertanum* (Poli), and *P. subtruncatum*, whereas during long periods of anoxia, rates of only 1 to 2 beats per minute were often recorded. Sometimes no beats could be detected at all suggesting existence of even longer pauses. However, because of the imprecision of the method these results must be considered preliminary at present.

SURVIVAL TIME IN CLOSED VIALS

The experimental temperatures in autumn and early winter were chosen to be near ambient levels (Fig. 1) to reveal the actual capabilities of these species to survive winter anoxia in their specific habitat and to observe the develop-

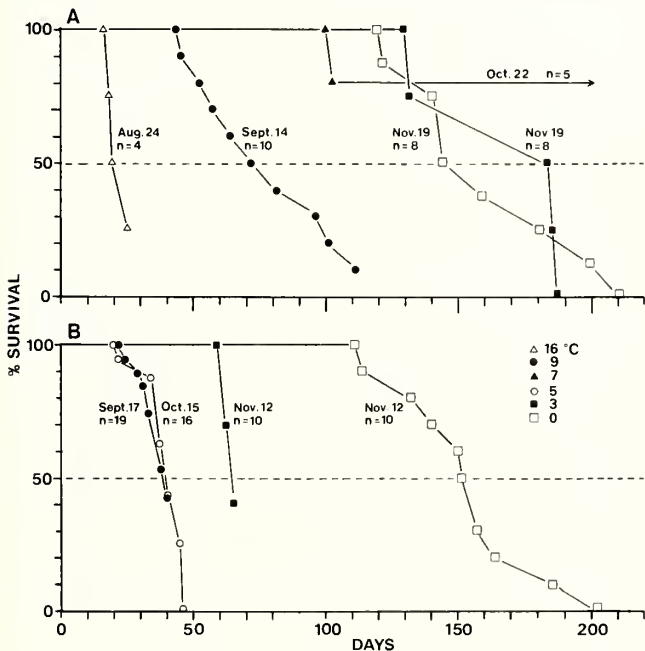


Fig. 2. An example of the survivorship curves of *Sphaerium corneum* (A) and *Pisidium amnicum* (B) in anoxia at different temperatures during autumn (August-November). The Oct. 22 experiment was interrupted after 201 days, when 4 of 5 clams were still alive. Numbers (n) denote individuals.

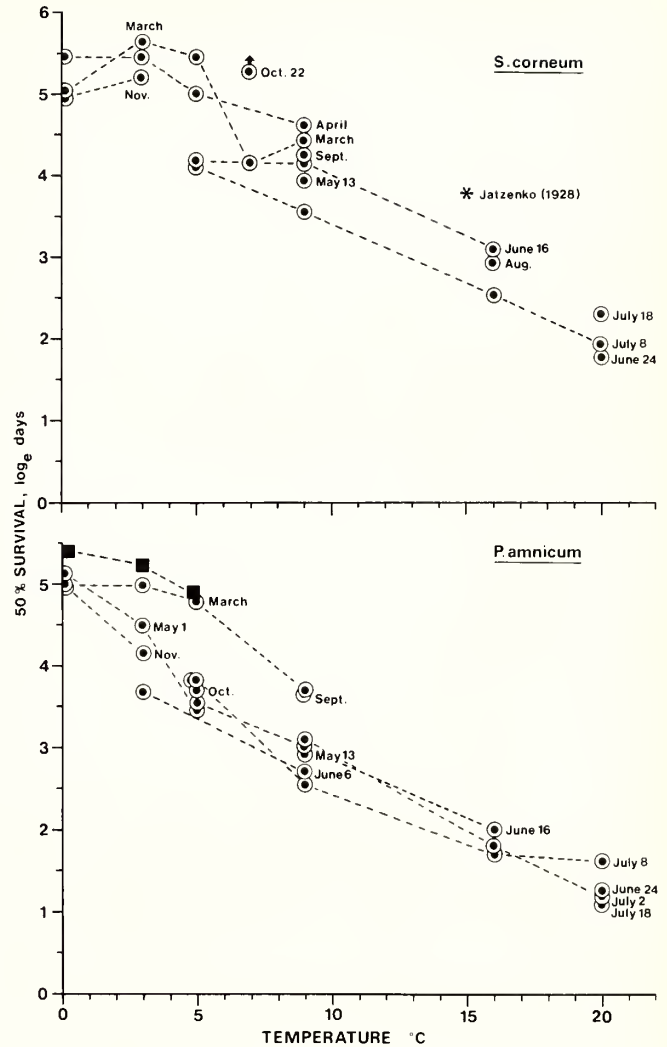


Fig. 3. The temperature dependence of the 50% survival time in experimental anoxia of *Sphaerium corneum* (A) and *Pisidium amnicum* (B). Results from the same sampling dates are connected by broken lines. Natural temperatures for each date are given in figure 1. The squares denote clams from Lake Pääjärvi collected in March 1985.

ment of the anoxic capacity. *Sphaerium corneum* survived almost 3 weeks at 16°C in August (Fig. 2A; further reference to time periods mean 50% survival). In September (at 9°C) this species was able to survive 72 days and in October-November as long as 150 days or more at temperatures of 7°C or below. The seasonal variation in temperature dependence of survival time can be seen in figure 3.

At 9°C the 50% survival time of *Pisidium subtruncatum* from Lake Esrom was 32 days (juveniles) and 36 days (adults; one adult survived 80 days). The single juvenile of *P. casertanum* died after 54 days whereas all three adults were alive when checked after 85 days of anoxia.

WINTERING IN ICE

The smaller ice block contained 4 living and 1 dead

Pisidium amnicum (73 individuals per m²) whereas the larger contained 18 living specimens (113 per m²).

GLYCOGEN CONTENT

The annual variation in total glycogen content is about 4-fold in both species (Fig. 4). Significant increases occurred in autumn (August - November) and in spring but decreases were observed during winter and summer.

The glycogen values for *Pisidium amnicum* thawed from the ice or taken from the shallow shore (0.2 m) are consistently lower (significantly in May, early June and July, analysis of variance, $P < 0.001$), than those collected only ca. 5 m apart from the depth of 1 m. The former probably overwintered in anoxic conditions in ice or frozen sediment (see below) and the latter in aerobic water. The same species from oligotrophic Lake Pääjärvi had a much lower glycogen content in March but showed an equal increase in early summer and a decrease in mid-summer.

There are some differences in the glycogen content between *Sphaerium corneum* from the outlet ditch and from

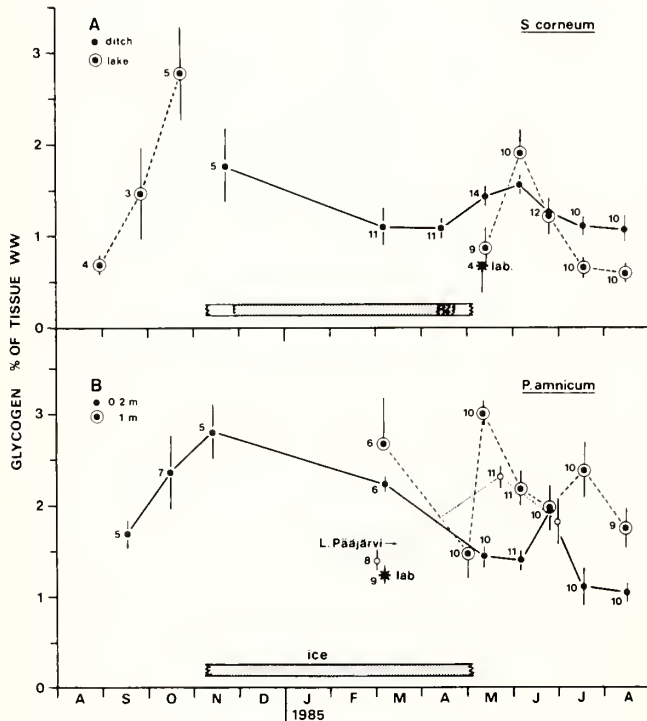


Fig. 4. The seasonal dynamics of total glycogen content (% of tissue wet weight, WW) in *Sphaerium corneum* (A) and *Pisidium amnicum* (B). Part of the samples in (A) came from lake littoral (0.5 m) and part from the outlet ditch (0.5 m, ca. 50 m from the lake). The asterisk shows the glycogen content of clams sampled on 22 October 1984 after 201 days of laboratory anoxia at 7°C. In the ice-cover the shaded period in A refer to the outlet ditch (partial ice-cover in spring). Figure (B) includes samples from two depths in the Siilaisempuro River (clams in the March sample from 0.2 m were frozen in ice) and three samples from the littoral (0.5 m) of Lake Pääjärvi, southern Finland. The asterisk shows glycogen content after 114 days at 3°C in a small volume of water in the laboratory (November sample in open bottle).

the lake. In general, the seasonal variation seems to be more prominent (peaks are higher and minima lower) in the lake littoral, which probably had longer period of oxygen depletion than the outlet ditch. The differences between October (lake) and November (ditch) as well as the June values are not significant whereas in May and July the glycogen content of the ditch clams is significantly higher (ANOVA, $P < 0.001$).

Histochemical techniques revealed a large deposit of glycogen granules in the subepithelial tissue of the foot in both species and in the mantle of *Sphaerium corneum* during winter. Some glycogen can also be seen in the gill (Figs. 5 and 6). On 1 May, five large specimens of *Pisidium amnicum* were relaxed by pentobarbital (Meier-Brook, 1976), the soft tissue was dissected into five different components and the glycogen content of each component was determined after drying at 60°C for 12 hrs. The glycogen contents were as following: foot 13.6% of tissue DW, mantle 12.7%, gills 11.9%, digestive diverticulae 4.3% and the rest 11.9%, yielding a weighted mean of 11.1%. The coincident mean value determined as percent of WW (1.47 ± 0.261) was in agreement. The content of the foot is not much higher than the other components probably because of the relatively low overall content at that time (the glycogen content in Fig. 6 is twice as high).

DISCUSSION

The most common response to anoxia is inactivity, including prominent bradycardia (e.g. De Zwaan, 1977). According to Gale (1976), heart rate in *Sphaerium transversum* slows down to "only a few times a minute" in anoxia, with which my results agree. Lowered metabolic rate, down to 5-10% of aerobic levels, is generally thought to be necessary in anoxia in order to save energy stores, because of the inefficiency of anaerobic metabolism (Zs. -Nagy, 1973; Gnaiger, 1983). The upward crawling response exhibited in this study by *S. corneum* would, in its habitat among aquatic vegetation (e.g. Zhadin, 1952), be advantageous in avoiding anoxia, although it might also increase the risk of predation. It is not known if this species naturally overwinters up out of the sediment, with the shells fixed by slime threads to aquatic macrophytes. A similar secretion of slime threads has been previously described [e.g., Zhadin (1952) and Ellis (1978)].

The survival times given here must be considered as minima because of the probable adverse effects of the small-volume closed-bottle method used. The accumulation of metabolites, H₂S and the effects of decaying specimens probably reduced survival even at low temperatures, although H₂S is often also present in nature. In this study the recovery of the individuals with closed shells was almost 100%, when measured by the ability to begin locomotion after transfer to aerobic water. This ability, however, would not guarantee the survival of the exhausted clams under natural conditions. Yet the survival times given here equal or exceed many of the scattered values given previously for molluscs and are of great enough length to have significant ecological implications.

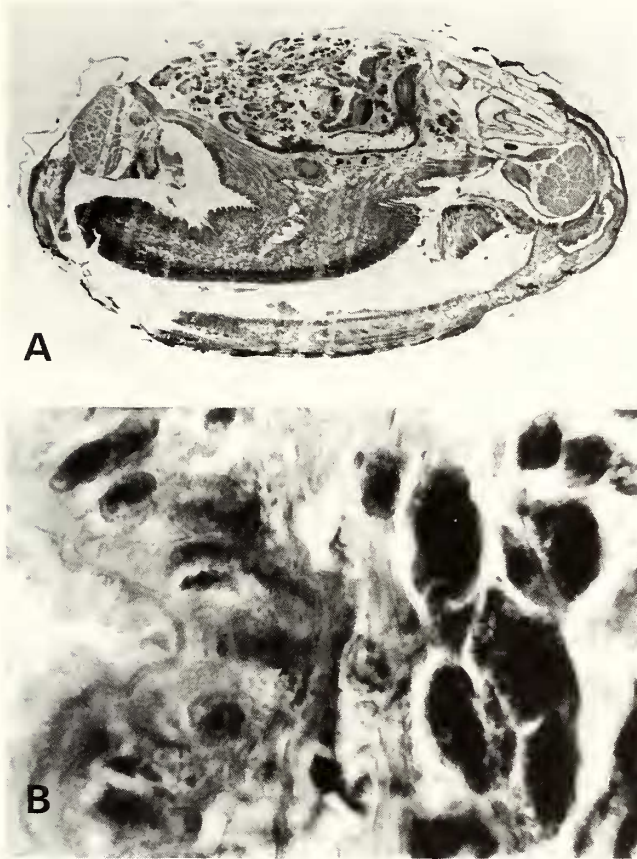


Fig. 5. (A) A median section of *Pisidium amnicum* collected from Siilaisempuro on 5 March 1985. High concentrations of glycogen in the subepithelial tissue of the foot is shown by black color. Allochrome HFW = 5.5 mm. (B) Ventral surface the foot of *P. amnicum* showing a epithelial cilia and subepithelial cells filled with glycogen granules. Allochrome HFW = 0.05 mm.

Survival times of up to 55 days at 10°C have been reported for marine molluscs (Theede *et al.*, 1969; Hammen, 1976). Zs.-Nagy (1973) gives 7-10 days as the anoxia tolerance period for *Anodonta cygnea* (L.) at 15°C; *Ligumia subrostrata* (Say), another freshwater species, survived more than 15 days at 25°C (Dietz, 1974). These are, however, short times when compared to aerial survival of one year or more of some tropical unionids at very high temperatures (Dance, 1958).

I also held a juvenile *Anodonta piscinalis* Nilsson (= *A. cygnea*) (30 mm, caught in March from Lake Pääjärvi), 66 days in anoxic water at 3°C. When transferred to aerobic water, the foot was soon introduced but started to withdraw upon touching only after 1 day.

Indications of the survival of Pisidiidae during anoxia in natural lakes range from 2-3 months (Juday, 1908; Holopainen and Jónasson, 1983) to 5-7 months (Eggleton, 1931). In addition, some experimental data are given by Juday (1908), Jatzenko (1928) and Eggleton (1931). My data on *Sphaerium corneum* closely agree with the 46-day survival

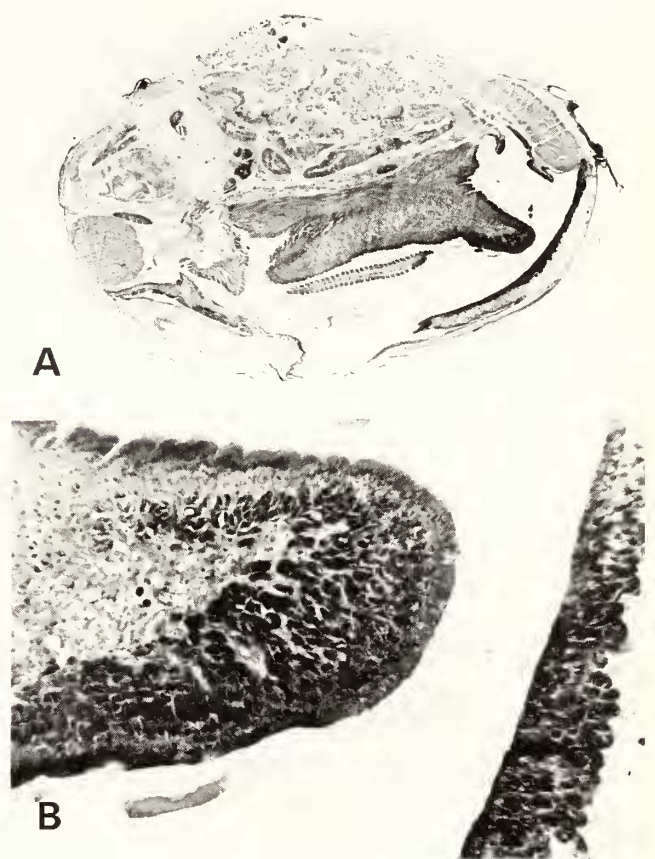


Fig. 6. A median section of *Sphaerium corneum* collected from Lake Varaslampi on 5 March 1985. Glycogen is seen as prominent black areas in both the foot tip and mantle with some reaction in the gill, also. Allochrome (A) HFW = 7 mm and (B) HFW = 0.7 mm.

time at 14-16°C reported by Jatzenko (1928) (see Fig. 3).

Besides temperature, survival times in anoxia probably depend on season, animal size and physiological state, as well as the possible existence of poisonous compounds (like H₂S) in the water. In my experiments the existence of H₂S was often suggested by black coloration on the shells and the odor emitted when the vials were opened. Zhadin (1952) reports *Sphaerium corneum* to be resistant to H₂S and to survive 14 days at 30mg/l of H₂S. Theede *et al.* (1969) and Shumway *et al.* (1983) have shown the deleterious effect of this compound on survival times of marine invertebrates.

Since only adults were used in most experiments, the effect of size could be examined only in case of *Pisidium casertanum* and *P. subtruncatum* from Lake Esrom. In both species juveniles appeared to die first. However, survival times of these species were long considering that at the time of sampling (September) they had already survived several weeks of anoxia in Lake Esrom (Holopainen and Jónasson, 1983).

The effects of temperature on survival times are prominent and appear linear on semi-log scale (Fig. 3). The average 50% survival time of *Pisidium amnicum*, which is

about 4.5 days at 20°C, increases up to 200 days at 0°C. The survival times of *Sphaerium corneum* are roughly twice as long at all temperatures. This seems to be in accordance with the habitat choice of these species. *P. amnicum* is an inhabitant of sandy bottoms of large lakes and prefers flowing water whereas *S. corneum* prefers muddy bottoms in small ponds and more nutrient-rich rivers (the Siilaisempuro River also has a sparse population of *S. corneum*).

In this survey the effects of season (seasonal changes in the physiological state of the clams) can not be clearly separated from the effects of temperature alone. In figure 3, however, some difference in survival ability between winter and summer is obvious and is probably a reflection, in part, of the seasonal changes in enzyme activity patterns and carbohydrate or lipid store dynamics. The temperature dependence could be influenced by the method, if the accumulation of metabolites in water is the main cause of death. At high temperatures the deleterious levels will soon be achieved in a small bottle.

Anoxic energy metabolism is based entirely on carbohydrates and stores of glycogen are a necessary prerequisite for sustained life without oxygen. This energy deposit, however, is probably not limiting and the seasonal dynamics appear in many species to be connected to other activities (growth, reproduction) rather than anoxia tolerance (De Zwaan, 1977; Zs.-Nagy and Galli, 1977; Dietz and Stern, 1977; Zandee *et al.*, 1980). However, in my results a clear depletion of stores is seen during winter, especially in anoxic conditions (Fig. 4).

The results of Zandee *et al.* (1980) on *Mytilus* show a high glycogen content (30-35% of DW) during the entire winter and a rapid decrease to 5% just before spawning in April. The dynamics of lipid content were reversed. Zandee *et al.* (1980) found highest concentrations of glycogen from the mantle and the "rest" (including the foot), whereas De Zwaan and Zandee (1973) reported only low values for the foot and muscles (half of that found in the mantle). My results for *Sphaerium corneum* resemble the dynamics of carbohydrate content of *S. transversum* (Dietz and Stern, 1977) by having a similar range (4 x) and a peak value in November; the glycogen content of *S. corneum* is however, ca. 50% higher.

In addition to overwinter glycogen consumption, the dynamics in glycogen content in pisidiids probably depends on the seasonal cycle of growth and reproduction as well. The population dynamics of *Sphaerium corneum* in Lake Varaslampi is not known but *Pisidium amnicum* in the Siilaisempuro River gives birth in July and new eggs are laid in August, but the embryos stay small until the following May. The increase in glycogen content in spring coincides with increase in oxygen, temperature and food availability as well as the start of both adult and embryo growth again. The drop in mid-July could be due to the release of embryos at that time. In *Mytilus* the carbohydrate metabolism is replaced by lipid metabolism in midsummer (Zandee *et al.*, 1980) but this has not been shown in pisidiids. In late summer and autumn the rebuilding of winter stores is again seen as an increase of glycogen.

Wintering within ice requires cold-hardiness even with the insulation of snow (about 50 cm) and ice. The long period of exceptionally cold weather (mean monthly air temperatures in January and February 1985 in Joensuu were -21.2° and -19.8°C, respectively) must have lowered the temperatures inside ice well below zero. However, *Pisidium* spp. have been shown to tolerate subzero temperatures, e.g. after 4 months at an experimental temperature of -4°C, the survival of *Pisidium* spp. was 57% (Olsson, 1981). The overwintering abilities of *Pisidium* and many other invertebrates in ice has long been known (Nordenskiöld, 1897, Grimås, 1961, Holmquist, 1973) but the quantitative importance of it has been only recently understood (Olsson, 1981).

Ice provides a refuge from predation, which in some cases may more than compensate for the risk of fatal freezing. In the Siilaisempuro River, probably more than half of the total *Pisidium amnicum* population live in the shallow areas and is susceptible to freezing.

In spite of limitations set by the simple method (small volume of closed bottle, no acclimation, exact clam volume/water volume ratio unknown) the results of this survey emphasize the importance of anaerobiosis for these species. The survival times are long enough to allow 6 months anoxic wintering and even at 20°C the 5-10 days survival times allow considerable distances to be covered in passive dispersal.

Presently the capacity for anaerobiosis of molluscs (including Pisidiidae), as well as the physiological basis of this ability, are much better known than the ecological consequences. For example, metabolic rates of molluscs in severe hypoxia can be greatly suppressed (down to 5-10% of normal, e.g. De Zwaan, 1977), and even in the presence of oxygen, the contribution of anaerobic metabolism to total energy yield can be considerable (e.g. Famme *et al.*, 1981). I suggest that these facts should be more seriously considered in all energetic studies on molluscs, especially in productive habitats that have great daily and/or seasonal variation in water oxygen pressure.

The two species of the present study seem to use their capacity for anaerobiosis only in order to tolerate the anoxic periods between more favorable conditions. Interestingly, a case of self-induced anaerobiosis (Taylor, 1976) and even obviously anoxic modes of life (Thomas, 1963, 1965; Way *et al.*, 1980; Shumway *et al.*, 1983) have been reported for some bivalves. In the latter cases productive environments and completely different behavioural responses are needed to ensure sufficient food intake for requirements set by elevated rate of glycolytic processes with their low efficiency in energy conversion.

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